

**PCT**WORLD INTELLECTUAL PROPERTY ORGANIZATION  
International Bureau

## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification <sup>6</sup> :</b> <b>A61K 38/20, 39/395</b>	<b>A1</b>	<b>(11) International Publication Number:</b> <b>WO 95/24918</b> <b>(43) International Publication Date:</b> 21 September 1995 (21.09.95)
<b>(21) International Application Number:</b> PCT/US95/02550 <b>(22) International Filing Date:</b> 7 March 1995 (07.03.95) <b>(30) Priority Data:</b> 08/212,629 14 March 1994 (14.03.94) US <b>(71) Applicant:</b> GENETICS INSTITUTE, INC. [US/US]; 87 CambridgePark Drive, Cambridge, MA 02140 (US). <b>(72) Inventors:</b> LEONARD, John, P.; 111 Heather Circle, Auburn, NH 03032 (US). GOLDMAN, Samuel; 9 Mohawk Drive, Acton, MA 01720 (US). O'HARA, Richard, Jr.; 65 Tyler Street, Quincy, MA 02170 (US). <b>(74) Agent:</b> BROWN, Scott, A.; Genetics Institute, Inc., 87 CambridgePark Drive, Cambridge, MA 02140 (US).		<b>(81) Designated States:</b> AU, CA, JP, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).  <b>Published</b> <i>With international search report.</i>
<b>(54) Title:</b> USE OF IL-12 AND IL-12 ANTAGONISTS IN THE TREATMENT OF AUTOIMMUNE DISEASES  <b>(57) Abstract</b>  Method of treating autoimmune conditions are disclosed comprising administering to a mammalian subject IL-12 or an IL-12 antagonist. In certain preferred embodiments the autoimmune condition is one which is promoted by an increase in levels of IFN- $\gamma$ or TNF- $\alpha$ . Suitable conditions for treatment include multiple sclerosis, systemic lupus erythematosus, rheumatoid arthritis, autoimmune pulmonary inflammation, Guillain-Barre syndrome, autoimmune thyroiditis, insulin dependent diabetes melitis and autoimmune inflammatory eye disease.		

**FOR THE PURPOSES OF INFORMATION ONLY**

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	GB	United Kingdom	MR	Mauritania
AU	Australia	GE	Georgia	MW	Malawi
BB	Barbados	GN	Guinea	NE	Niger
BE	Belgium	GR	Greece	NL	Netherlands
BF	Burkina Faso	HU	Hungary	NO	Norway
BG	Bulgaria	IE	Ireland	NZ	New Zealand
BJ	Benin	IT	Italy	PL	Poland
BR	Brazil	JP	Japan	PT	Portugal
BY	Belarus	KE	Kenya	RO	Romania
CA	Canada	KG	Kyrgyzstan	RU	Russian Federation
CF	Central African Republic	KP	Democratic People's Republic of Korea	SD	Sudan
CG	Congo	KR	Republic of Korea	SE	Sweden
CH	Switzerland	KZ	Kazakhstan	SI	Slovenia
CI	Côte d'Ivoire	LI	Liechtenstein	SK	Slovakia
CM	Cameroon	LK	Sri Lanka	SN	Senegal
CN	China	LU	Luxembourg	TD	Chad
CS	Czechoslovakia	LV	Latvia	TG	Togo
CZ	Czech Republic	MC	Monaco	TJ	Tajikistan
DE	Germany	MD	Republic of Moldova	TT	Trinidad and Tobago
DK	Denmark	MG	Madagascar	UA	Ukraine
ES	Spain	ML	Mali	US	United States of America
FI	Finland	MN	Mongolia	UZ	Uzbekistan
FR	France			VN	Viet Nam
GA	Gabon				

Use of IL-12 and IL-12 Antagonists  
in the Treatment of Autoimmune Diseases

Background of the Invention

Gamma interferon (IFN- $\gamma$ ) and tumor necrosis factor-alpha (TNF- $\alpha$ ) have been implicated in the development, exacerbation and/or recurrence of numerous autoimmune conditions. For example, both IFN- $\gamma$  and TNF- $\alpha$  have been associated with the course of multiple sclerosis [Choflon et al., Eur. Cytokine Netw. 3(6), 1992, pp. 523-531; Steinman, Scientific American, September 1993, pp. 107-114; Hofman et al., J. Exp. Med. 170, 1989, pp. 607-612; Panitch et al., Neurology, 37, 1987, pp. 1097-1102] and Type-I diabetes (insulin-dependent diabetes melitis, IDDM) [Castano et al., Annu. Rev. Immunol. 8, 1990, pp. 647-679; Campbell et al., J. Clin. Invest. 87, 1991, pp. 739-742]. While TNF- $\alpha$  has been found to promote development of rheumatoid arthritis [Feldmann et al., Progress in Growth Factor Research, 4, 1992, pp. 247-255], administration of IFN- $\gamma$  has been linked to improvements in arthritic subjects [Veys et al., J. Rheumatology, 15(4), 1988, pp. 570-574]. Studies have also demonstrated the involvement of IFN- $\gamma$  in the autoimmune diseases processes associated with systemic lupus erythematosus (SLE) [Funauchi et al., Tohoku J. Exp. Med., 164, 1991, pp. 259-267; Bankhurst, J. Rheumatology, 14(supp. 13), 1987, pp. 63-67], autoimmune thyroiditis [Tang et al., Eur. J. Immunol. 23, 1993, pp. 275-278], and autoimmune inflammatory eye disease (e.g., autoimmune uveoretinitis) [Charteris et al., Immunology 75, 1992, pp. 463-467]. Development of autoimmune pulmonary inflammation [Deguchi et al., Clin. Exp. Immunol. 85, 1991, pp. 392-395] and Guillain-Barre syndrome

[Baron et al., Proc. Natl. Acad. Sci. USA 90, 1993, pp. 4414-4418] have also been tied to TNF- $\alpha$  activity.

Interleukin-12 (IL-12) is a heterodimeric cytokine which was originally identified as a factor which induces IFN- $\gamma$  from T cells and natural killer cells as set forth in PCT/US91/06332, published April 2, 1992. PCT/US91/06332 refers to IL-12 as Natural Killer Cell Stimulating Factor or NKSF. EP 433827, published June 26, 1991 discloses IL-12 as a cytotoxic lymphocyte maturation factor (CLMF). IL-12 also stimulates natural killer cells *in vitro* by increasing their ability to lyse target cells at a level comparable to that obtained with IFN- $\alpha$  and IL-2, well-known activators of natural killer cells' cytotoxic activity. Additional *in vitro* activities of IL-12 which have been identified include induction of TNF- $\alpha$ ; induction of T cell proliferation as a co-stimulant; suppression of IL-2 induced proliferation of natural killer blasts; suppression of IL-2 induced proliferation of T cell receptor- $\gamma\delta$ -positive cells; promotion of Th1 T cell differentiation from progenitors; enhancement of Th1, but not Th2 proliferation; enhancement of T cell cytolytic activity; enhancement of cytotoxic lymphocyte generation; enhancement of natural killer and natural killer blast cytolytic activity; *ex vivo* enhancement of natural killer activity in peripheral blood mononuclear cells of IL-2-treated patients; induction of adhesion molecules on natural killer cells; induction of perforin and granzyme B mRNAs in natural killer blasts; induction of IL-2 receptor subunits (p55, p75) on natural killer cells; suppression of IgE synthesis by IFN- $\gamma$ -dependent and independent mechanisms; modulation of T cell development in fetal thymic organ cultures; and synergy with kit ligand to promote growth of myeloid and B cell progenitors. The known *in vivo* activities of IL-12 include induction of IFN- $\gamma$ ; enhancement

of natural killer cell activity in spleen, liver, lungs and peritoneal cavity; enhancement of generation of allo-specific cytotoxic lymphocytes; induction of extramedullary hematopoiesis in mouse spleen; reversible suppression of hematopoiesis in bone marrow; reversible induction of anemia, lymphopenia, and neutropenia in mice; suppression of anti-IgD induced IgE, IgG1, and IL-4 expression; increased survival in SCID mice treated with *Toxoplasma gondii*; cure of leishmaniasis in susceptible strains of mice; decreased bioburden in cryptococcoses model; suppression of tumor growth; and promotion of immunity to tumor cells. IL-12 is also induced *in vivo* in the shwarzman reaction model of septic shock.

Although IL-12 can induce production of IFN- $\gamma$  and TNF- $\alpha$  *in vivo*, the relationship of *in vivo* levels of IL-12 to autoimmune diseases which are affected by levels of IFN- $\gamma$  and TNF- $\alpha$  has not been established. Furthermore, the effects of administration of IL-12 or antagonists of endogenous IL-12 (such as anti-IL-12 antibodies) on autoimmune diseases associated with induction of IFN- $\gamma$  or TNF- $\alpha$  have not been examined.

#### 15     Summary of the Invention

The present invention provides methods of treating (e.g., curing, ameliorating, delaying or preventing onset of, preventing recurrence or relapse of) autoimmune conditions or diseases. In preferred embodiments, the condition is one promoted by an increase in levels of a cytokine selected from the group consisting of TNF- $\alpha$  or IFN- $\gamma$ . Such conditions include, without limitation, those selected from the group consisting of multiple sclerosis, systemic lupus erythematosus, rheumatoid arthritis, autoimmune pulmonary inflammation, Guillain-Barre syndrome, autoimmune thyroiditis, insulin dependent diabetes melitis and

autoimmune inflammatory eye disease. Multiple sclerosis and insulin-dependent diabetes melitis are particularly preferred conditions for treatment in accordance with the present invention as described herein.

5 In certain embodiments the method of treatment of the present invention comprises administering to a mammalian subject a therapeutically effective amount of an IL-12 antagonist, preferably an antibody or other species which is immunoreactive with IL-12. in certain preferred embodiments the IL-12 antagonist is administered in a dose of about 0.05 to about 25 mg/kg, preferably of about 0.2 to about 2 mg/kg. The antagonists can also be administered in combination with a pharmaceutically acceptable carrier.

10 In other embodiments, the method of treatment of the present invention comprises administering to a mammalian subject a therapeutically effective amount of IL-12. In certain embodiments, the IL-12 may be administered in a dose of about 0.001 to about 1000  $\mu\text{g/kg}$ , preferably about 0.01 to about 100  $\mu\text{g/kg}$ . The IL-12 can also be administered in combination with a pharmaceutically acceptable carrier.

15

#### Brief Description of the Figures

Fig. 1 presents graphs of data relating to the adoptive transfer of experimental allergic encephalomyelitis (EAE) using lymph node and spleen cells stimulated in vitro with PLP and rmIL-12. Spleens and Lymph nodes were harvested from mice 10 days after  
20 immunization with PLP and stimulated in vitro with antigen alone (open symbols) or antigen and 20ng/ml rmIL-12 (closed symbols) as described in materials and methods. Disease was transferred using  $30 \times 10^6$  cells. The results are presented as mean score for (a) lymph nodes

(n=7) and (b) spleen cells (n=5). The data is representative of at least two separate experiments. See example 1.

Fig. 2 presents graphs of data relating to IFN- $\gamma$  and TNF- $\alpha$  production from LNC stimulated in vitro with PLP and IL-12. LNC ( $2.5 \times 10^6$ /ml) from PLP immunized mice were cultured with PLP alone, PLP and rmIL-12 (20ng/ml) or PLP, rmIL-12 and anti-IFN- $\gamma$  (5 $\mu$ g/ml) for 96 hours prior to cell transfer with  $30 \times 10^6$  cells. (a) IFN- $\gamma$  and TNF- $\alpha$  measured by ELISA in the supernatants of pooled cultures. (b) Mean disease score after the transfer of stimulated lymph node cells. n=3 for PLP alone and PLP + IL-12 and n=4 for PLP + IL-12 + anti-IFN- $\gamma$ . See example 1.

Fig. 3 depicts graphs of data relating to the effects of In vivo administration of IL-12 on the adoptive transfer of EAE using PLP stimulated LNC. LNC from PLP immunized mice were cultured in vitro with antigen as described in materials and methods and transferred to naive mice. rmIL-12 (0.3 $\mu$ g/mouse) was administered on days 0, 1 and 2 after cell transfer (closed circles) and mice monitored for signs of disease. Control mice received and equal volume of saline (open circles). (a) Mean clinical score following the transfer of  $30 \times 10^6$  LNC cells (n=5). (b) Mean clinical score following the transfer of  $10 \times 10^6$  LNC (n=4). Figure 3a is representative of three separate experiments. See example 1.

Fig. 4 depicts graphs of data relating to the effects of in vivo administration of anti-IL-12 antibody on the adoptive transfer of EAE using PLP stimulated LNC. LNC from PLP immunized mice were cultured in vitro with antigen as described in materials and methods and  $30 \times 10^6$  cells transferred to naive mice. Anti-IL-12 antibody (sheep anti-mouse

polyclonal antibody, 200 $\mu$ g/ mouse) was administered by intraperitoneal injection starting on the day of cell transfer (closed circles). Control mice received an equivalent amount of sheep IgG (open circles). (a) Mean clinical score following administration of  $\alpha$ IL-12 antibody everyother day from day 0 to day 6. (b) Mean clinical score following administration of  $\alpha$ IL-12 antibody everyother day from day 0 to day 12. (n=5-7). See example 1.

Figs. 5 and 6 present graphs of data relating to disease incidence in NOD mice upon administration of IL-12. See example 2.

#### 10 Detailed Description

The present invention provides methods for treating autoimmune conditions. "Autoimmune conditions" are those in which the subject's own immune system reacts against the subject's cells or tissues, resulting in damage to those cells or tissues. A particular autoimmune condition is "promoted by an increase in levels of a cytokine" when a increase in serum or tissue levels of such cytokine can cause or contribute to the development or recurrence of, or to the acceleration of the onset of, such autoimmune condition.

Autoimmune conditions which are promoted by an increase in levels of IFN- $\gamma$  and/or TNF- $\alpha$  include, without limitation, multiple sclerosis, systemic lupus erythematosus, rheumatoid arthritis, autoimmune pulmonary inflammation, Guillain-Barre syndrome, autoimmune thyroiditis, insulin dependent diabetes melitis and autoimmune inflammatory eye disease.

"IL-12 antagonists" include (1) species that will bind IL-12 or biologically active fragments thereof, and (2) species that will interfere with the binding of IL-12 to



receptors or other binding proteins. Antagonists that bind IL-12 include, without limitation, antibodies (mono- or polyclonal) and fragments thereof (including F<sub>ab</sub> fragments), chimeric antibodies and fragments thereof, lectins, IL-12 receptors or fragments thereof, reactive peptides or fragments thereof, and organic small molecules designed to mimic the bioactivity of IL-12 receptors. Antagonists that interfere with IL-12 binding include, without limitation, chemically or genetically modified peptides of IL-12, subunits of IL-12 and fragments thereof, homopolymers of IL-12 subunits and fragments thereof, and organic small molecules designed to mimic the bioactivity of IL-12. Preferably, antagonists that interfere with IL-12 binding interfere with its binding to receptors which induce IFN- $\gamma$  or TNF- $\alpha$ , without inducing the same level of such factors as would binding of IL-12 to the receptor.

IL-12 antagonists can be produced by methods well known to those skilled in the art. For example, monoclonal IL-12 antibodies can be produced by generation of antibody-producing hybridomas in accordance with known methods (see for example, Goding. 1983. Monoclonal antibodies: principles and practice. Academic Press Inc., New York; Yokoyama. 1992. "Production of Monoclonal Antibodies" in Current Protocols in Immunology. Unit 2.5. Greene Publishing Assoc. and John Wiley & Sons). Polyclonal sera and antibodies to IL-12 can be produced by inoculation of a mammalian subject with IL-12 or fragments thereof in accordance with known methods. Chizzonite et al., J. Immunol. 148, 1992, p. 3117, describes the identification and isolation of an IL-12 receptor. Fragments of antibodies, receptors or other reactive peptides can be produced from the corresponding antibodies by cleavage of and collection of the desired fragments in

accordance with known methods (see for example, Goding, supra; Andrew et al. 1992. "Fragmentation of Immunoglobulins" in Current Protocols in Immunology. Unit 2.8. Greene Publishing Assoc. and John Wiley & Sons). Chimeric antibodies may also be produced in accordance with known methods.

5                   In methods of the present invention using IL-12, any form of IL-12 may be used, so long as that form of IL-12 is capable of treating the desired autoimmune condition. For example, IL-12 may be in the form of the heterodimer comprised of a 40 kD subunit disulfide-bonded to a 35 kD subunit. When IL-12 is a heterodimer, the 40 kD subunit has substantial homology to the 40 kD subunit of human IL-12 as set forth in PCT/US91/06332  
10                   and is disulfide bonded to a 35 kD subunit having substantial homology to the 35 kD subunit of human IL-12 as set forth in that same PCT publication. "Substantial homology" means greater than 75% homology at the amino acid level, while retaining the ability to treat the desired autoimmune condition in a mammalian subject. Another form of IL-12 which may be used in the present invention is an IL-12 subunit capable of treating the desired  
15                   autoimmune condition in a mammalian subject. Such an IL-12 40 kD subunit has substantial homology to the human IL-12 40 kD subunit disclosed in PCT/US91/06332, and such an IL-12 35 kD subunit has substantial homology to the human IL-12 35 kD subunit disclosed in such PCT publication. Fragments of the IL-12 subunits that retain IL-12 biological activity are also be useful to treat autoimmune conditions in mammalian subjects, in accordance with  
20                   the present invention.

For use in the present invention, it is preferable to produce IL-12 recombinantly, through expression of DNA sequences encoding one or both of the IL-12

subunits in a suitable transformed host cell. For example, using known methods the DNA sequences encoding human IL-12 set forth in PCT/US91/06332 may be linked to an expression vector such as pED (Kaufman et al., Nucleic Acids Res. 19, 4484-4490(1991)). In such an expression vector, sequences which optimize translation such as CCACC (Kozak, 5 M., Nucleic Acids Res. 12, 857-871 (1984)) may be added 5' to the initiation codon using known methods. The expression vector containing the IL-12 subunits may then be transformed into a host cell, and protein expression may be induced and maximized, to produce heterodimeric human IL-12. For production of heterodimeric IL-12, the DNA sequences encoding the IL-12 subunits may be present on different expression plasmids or 10 present in tandem on a single expression plasmid.

When a subunit or fragment of IL-12 is used to practice the present invention, it may also be produced recombinantly using known methods. For example, the DNA sequence encoding the human IL-12 40 kD subunit set forth in PCT/US91/06332 may be linked to an expression vector, transformed into a host cell, and expression induced and 15 maximized to produce the human IL-12 40 kD subunit. Similarly, the DNA sequences encoding the human IL-12 35 kD subunit as set forth in the PCT publication may be linked to an expression vector, transformed into a host cell, and expression induced and maximized to produce the corresponding protein. Of course, degenerate DNA sequences encoding the IL-12 subunits may also be employed to produce IL-12 for use in the present invention, as 20 can DNA sequences encoding allelic variants of the IL-12 subunits. Chemically or genetically modified forms of IL-12 and its subunits can also be made in accordance with the methods disclosed in the PCT publication.

Any suitable expression vector may be employed to produce IL-12 for use in the present invention. For mammalian expression, numerous expression vectors are known in addition to the pED vector mentioned above, such as pEF-BOS (Mizushima et al., *Nucleic Acids Res.* 18, 5322 (1990)); pXM, pJL3 and pJL4 (Gough et al., *EMBO J.* 4, 645-653 (1985)); and pMT2 (derived from pMT2-VWF, A.T.C.C. #67122; see PCT/US87/00033).  
5 Suitable expression vectors for use in yeast, insect, and bacterial cells are also known. Construction and use of such expression vectors is well within the level of skill in the art.

Suitable host cells for recombinant production of IL-12 useful in the present invention include, for example, mammalian cells such as Chinese hamster ovary (CHO)  
10 cells, monkey COS cells, mouse 3T3 cells, mouse L cells, myeloma cells such as NSO (Galfre and Milstein, *Methods in Enzymology* 73, 3-46 (1981)), baby hamster kidney cells, and the like. IL-12 may also be produced by transformation of yeast, insect, and bacterial cells with DNA sequences encoding the IL-12 subunits, induction and amplification of protein expression, using known methods.

15 Recombinantly produced IL-12 can be purified from culture medium or cell extracts by conventional purification techniques. Culture medium or cell extracts containing IL-12 may be concentrated using a commercially available protein concentration filter, for example, an Amicon or Millipore Pellicon ultrafiltration unit. Following the concentration step, the concentrate can be applied to a purification matrix such as a gel filtration medium.  
20 Alternatively, an anion exchange resin can be employed, for example, a matrix or substrate having pendant diethylaminoethyl (DEAE) groups. The matrices can be acrylamide, agarose, dextran, cellulose or other types commonly employed in protein purification. Alternatively,

a cation exchange step can be employed. Suitable cation exchangers include various insoluble matrices comprising sulfopropyl or carboxymethyl groups. The purification of IL-12 from culture supernatant may also include one or more column steps over such affinity resins as lectin-agarose, heparin-toyopearl® or Cibacrom blue 3GA Sepharose®; or by  
5 hydrophobic interaction chromatography using such resins as phenyl ether, butyl ether, or propyl ether; or by immunoaffinity chromatography. Finally, one or more reverse-phase high performance liquid chromatography (RP-HPLC) steps employing hydrophobic RP-HPLC media, e.g., silica gel having pendant methyl or other aliphatic groups, can be employed to further purify IL-12 for use in the present methods and compositions. Some or  
10 all of the foregoing purification steps, in various combinations, can be employed to provide a substantially homogeneous isolated recombinant protein. Purification of IL-12 subunits or fragments for use in the present invention may differ from the optimal protocol for purification of the heterodimeric protein.

Preferably, when human IL-12 is produced recombinantly as set forth above, it  
15 may be purified by the following method. The cells in which the human IL-12 has been made may be removed from the conditioned medium by filtration, and the conditioned medium is loaded onto Q-Sepharose FastFlow™ (available from Pharmacia) or an equivalent anion exchange medium, which has been equilibrated in 10-30 mM Tris-HCl, pH 7.8-8.3. The column is then washed extensively with the same buffer followed by a wash with 30-45  
20 mM histidine, pH 5.1-5.8, followed by a wash with the original equilibration buffer. The recombinant human IL-12 is eluted from the column with a buffer containing 20-50 mM Tris-HCl, pH 7.8-8.5, and 0.15 to 0.50 M NaCl. The eluted material is loaded onto CM-

Sepharose FastFlow™ (available from Pharmacia) or equivalent cation exchange medium which has been equilibrated in 20-50 mM MES, pH 5.7-6.4, and washed extensively with the same buffer. The column is washed with a buffer containing 20-40 mM sodium phosphate, pH 6.8-7.5 and 0.2-0.5 M NaCl. The eluted material is concentrated using an Amicon™  
5 S1Y30 or equivalent spiral cartridge membrane which has been washed and equilibrated in the elution buffer used in the CM-Sepharose FastFlow™ column. The material is concentrated to approximately 5% of the column volume of the final chromatographic step, which is size exclusion using S200 Sephacryl™ (available from Pharmacia) or an equivalent size exclusion resin. The size exclusion column is equilibrated and eluted with phosphate  
10 buffered saline, pH 7.2-7, and the recombinant human IL-12 peak is collected and filtered for use in the method of the invention. Those of skill in the art of protein purification may use alternative purification methods to obtain recombinantly-produced human IL-12 for use in the method of the invention.

IL-12 may be purified from culture medium or extracts of cells which  
15 naturally produce the protein and used in the present invention. Exemplary purification schemes for naturally produced IL-12 are set forth in PCT/US91/06332 and in EP 433827.

Pharmaceutical compositions containing an IL-12 antagonist or IL-12 which are useful in practicing the methods of the present invention may also contain pharmaceutically acceptable carriers, diluents, fillers, salts, buffers, stabilizers and/or other  
20 materials well-known in the art. The term "pharmaceutically acceptable" means a material that does not interfere with the effectiveness of the biological activity of the active ingredient(s) and that is not toxic to the host to which it is administered. The characteristics

of the carrier or other material will depend on the route of administration.

It is currently contemplated that the various pharmaceutical compositions should contain about 0.1 micrograms to about 1 milligram per milliliter of the IL-12 antagonist or IL-12.

5 Administration can be carried out in a variety of conventional ways. Intraperitoneal injection is the preferred method of administration of the IL-12 antagonist or IL-12. Intravenous, cutaneous or sub-cutaneous injection may also be employed. For injection, IL-12 antagonist or IL-12 will preferably be administered in the form of pyrogen-free, parenterally acceptable aqueous solutions. The preparation of such parenterally  
10 acceptable protein solutions, having due regard to pH, isotonicity, stability and the like, is within the skill of the art.

The amount of IL-12 antagonist or IL-12 used for treatment will depend upon the severity of the condition, the route of administration, the reactivity of the IL-12 antagonist with IL-12 or the activity of the IL-12, and ultimately will be decided by the  
15 treatment provider. In practicing the methods of treatment of this invention, a therapeutically effective amount of an IL-12 antagonist or IL-12 is administered. The term "therapeutically effective amount" means the total amount of each active component of the method or composition that is sufficient to show a meaningful patient benefit (e.g., curing, ameliorating, delaying or preventing onset of, preventing recurrence or relapse of). One  
20 common technique to determine a therapeutically effective amount for a given patient is to administer escalating doses periodically until a meaningful patient benefit is observed by the treatment provider. When applied to an individual active ingredient, administered alone, the

term refers to that ingredient alone. When applied to a combination, the term refers to combined amounts of the active ingredients that result in the therapeutic effect, whether administered in combination, serially or simultaneously. A therapeutically effective dose of an IL-12 antagonist in this invention is contemplated to be in the range of about 0.05 mg/kg to about 25 mg/kg. A therapeutically effective dose of IL-12 in this invention is contemplated to be in the range of about 0.001 to about 1000  $\mu$ g/kg. The number of administrations may vary, depending on the individual patient and the severity of the autoimmune condition.

The IL-12 antagonist or IL-12 used in practicing the present invention may be administered alone or combined with other therapies for autoimmune conditions, such as steroidal or other anti-inflammatory therapies and administration of other cytokines.

The methods of the present invention are further described in the following examples, which are intended to illustrate the invention without limiting its scope.

15

#### Example 1

Experimental allergic encephalomyelitis (EAE) is a T cell mediated autoimmune disease of the central nervous system (CNS). Disease can be induced in susceptible strains of mice by immunization with CNS myelin antigens or alternatively, disease can be passively transferred to susceptible mice using antigen stimulated CD4<sup>+</sup> T cells [Pettinelli, J. Immunol. 127, 1981, p. 1420]. EAE is widely recognized as an acceptable animal model for multiple sclerosis in primates [Alvord et al. (eds.) 1984].



Experimental allergic encephalomyelitis - A useful model for multiple sclerosis. Alan R. Liss, New York]. The effects of administration of an IL-12 antagonist on induction of EAE following the adoptive transfer of lymphocytes from immunized mice restimulated *in vitro* with a synthetic peptide of myelin proteolipid protein (PLP).

5

#### Adoptive transfer of PLP sensitized LNC

Female SJL/J mice (7-10wks) were purchased from The Jackson Laboratory, housed 5 to a cage and fed standard rodent chow diet with water ad libitum. Mice were immunized in two sites on the flank with 150 $\mu$ g of mouse PLP peptide comprising residues 139-151 (provided by G Brown, Genetics Institute). PLP was administered in 200 $\mu$ l of Complete Freund's adjuvant containing 2mg/ml *Mycobacteria Tuberculosis* H37RA (Difco). On the day of immunization mice were injected intravenously with  $0.75 \times 10^{10}$  *Bordetella pertussis* bacilli (Massachusetts Public Health Laboratories, Boston, MA). Ten days after immunization, spleens and lymph nodes (popliteal, axillary and brachial) were harvested and the cells resuspended in RPMI-1640 containing 10% FBS (Hyclone),  $5 \times 10^{-5}$  M 2-Mercaptoethanol, 100 $\mu$ g/ml streptomycin and 100U/ml penicillin. PLP was added to the cultures at 2 $\mu$ g/ml. After 96 hours, the cells were harvested, washed twice and  $30 \times 10^6$  cells (either LNC or spleen) injected i.p. into naive SJL/J mice.

20

#### Clinical evaluation of disease

Mice were observed for clinical signs of EAE and scored on a scale of 0 to 3 as follows:

- 0.5 - Distal limp tail
- 1.0 - Complete limp tail
- 1.5 - Limp tail and hind limb weakness (unsteady gait)
- 2.0 - Partial hind limb paralysis
- 5      3.0 - Complete bilateral hind limb paralysis

**In Vitro Administration of IL-12 prior to cell transfer.**

Recombinant murine IL-12 (20ng/ml, rmIL-12, Genetics Institute) was added to the *in vitro* cultures of lymph node or spleen cells with antigen prior to cell transfer.

- 10      After 96 hours the cells were washed twice and  $30 \times 10^6$  cell transferred to naive SJL/J mice to determine the effects of IL-12 on the subsequent course of disease.

- 15      In separate experiments, LNC were cultured with either antigen alone, antigen plus IL-12 (20ng/ml) or antigen plus IL-12 plus a neutralizing antibody to IFN- $\gamma$  (5 $\mu$ g/ml from Endogen). At the end of the culture period supernatants were collected (pooled from three flasks) and IFN- $\gamma$  and TNF- $\alpha$  measured by ELISA (from Genzyme). 30 $\times 10^6$  cells from each group were transferred to naive mice which were monitored for signs of disease.

**In vivo Administration of IL-12 and Anti-IL-12 antibody following the transfer of PLP stimulated LNC**

- 20      rmIL-12 (0.3 $\mu$ g/mouse, 200 $\mu$ l i.p.) was administered to mice following the transfer of either  $30 \times 10^6$  or  $10 \times 10^6$  PLP stimulated LNC. IL-12 was administered on days 0, 1 and 2 following cell transfer. Control mice received an equal volume of vehicle alone. To determine if IL-12 is involved in the induction of disease following the transfer of PLP

stimulated LNC, mice were treated with 200 $\mu$ g of a sheep polyclonal antibody against murine IL-12 (200 $\mu$ l i.p.) on alternate days for either 6 or 12 days in total following cell transfer and the mice monitored for signs of disease. Control mice received an equal amount of sheep IgG. The mice were monitored.

5

#### Effect of rmIL-12 on restimulation of PLP primed T cells *in vitro*

LNC from mice immunized with PLP as described in methods were stimulated *in vitro* with antigen in the absence or presence of rmIL-12 (20ng/ml) for 96 hours after which time they were tested for their ability to transfer disease to naive SJL/J mice. Mice receiving LNC stimulated *in vitro* with PLP alone developed clinical signs of disease between days 6 and 8. All control mice reached scores of 2 or greater (7/7) with 4 out of 7 mice progressing to complete hind limb paralysis which lasted between 1 and 4 days (Fig. 1a). All the control mice had recovered by day 19. In contrast mice receiving cells cultured *in vitro* with PLP and IL-12 developed severe EAE with rapid onset of clinical signs (Fig. 1a). By day 6, 4 out of 7 mice had clinical scores of 2 or greater and all mice went on to develop full hind limb paralysis by day 8. In this particular experiment 5 out of 7 mice failed to recover from the paralysis.

Spleen cells from PLP immunized mice stimulated *in vitro* with antigen for 96 hours in the absence or presence of rmIL-12 (20ng/ml) were also examined to determine whether they could transfer disease to naive SJL/J mice. The severity of disease following the adoptive transfer of  $30 \times 10^6$  PLP stimulated spleen cells was mild compared to that induced by an equivalent number of PLP stimulated LNC, with only 2 out of 5 mice

developing complete hind limb paralysis and the remaining 3 mice displaying only mild signs of disease (Fig. 1b). Similar to the results observed with LNC, the addition of rmIL-12 (20ng/ml) to the in vitro culture of spleen cells prior to transfer exacerbated subsequent disease (Fig. 1b). Mice receiving spleen cells stimulated with PLP and rmIL-12 developed clinical signs of disease by day 6 and all progressed to full hind limb paralysis by day 12. The mean duration of paralysis in these mice was 5.4 days (range 2-8 days).

#### Cytokine production following in vitro stimulation of LNC with PLP and IL-12

To determine the effects of IL-12 on cytokine production during the in vitro stimulation with antigen, LNC from PLP primed mice were cultured with either PLP alone, PLP and IL-12 (20ng/ml) or PLP, IL-12 and a neutralizing anti-IFN- $\gamma$  antibody. At the end of the in vitro culture, IFN- $\gamma$  and TNF- $\alpha$  in the supernatant were measured by ELISA and the cells tested for their ability to transfer disease to naive mice. The addition of IL-12 during the in vitro stimulation of LNC with PLP resulted in a greater than 10 fold increase in IFN- $\gamma$  (5.2ng/ml control and 64ng/ml IL-12) and a two fold increase in TNF- $\alpha$  in the cell culture supernatant (Fig. 2a). The addition of a neutralizing antibody to IFN- $\gamma$  during the culture of LNC with antigen and IL-12 completely blocked IFN- $\gamma$  detection, but had no effect on the increase in TNF- $\alpha$  in the supernatants which remained approximately two fold higher relative to controls (100pg/ml controls compared to 180pg/ml with  $\alpha$ IFN- $\gamma$  antibody). Furthermore, transfer of the cells stimulated in vitro with PLP and IL-12 in the presence of a neutralizing antibody to IFN- $\gamma$  were still capable of inducing severe disease with the same kinetics and duration to that seen following the transfer of cells stimulated with PLP and IL-

12 alone (Fig. 2b).

#### The effect of in vivo administration of IL-12 on disease progression

Following the transfer of  $30 \times 10^6$  PLP stimulated LNC mice were administered  
5 rmIL-12 ( $0.3 \mu\text{g}/\text{mouse}$ ) or saline for 3 days and the effects on the subsequent course of  
disease monitored. The onset and progression of disease in the controls was similar to that  
described above with clinical signs evident between days 6-8 after the transfer of LNC with  
80% of the mice progressing to full bilateral hind limb paralysis. Peak disease in the  
control mice lasted approximately 3 days after which time the mice spontaneously recovered  
10 (Fig. 3a). Administration of rmIL-12 ( $0.3 \mu\text{g}/\text{mouse}$ ) for 3 days after the transfer of an  
equivalent number of primed LNC from the same in vitro cultures dramatically altered the  
course of disease. Although the time of onset of symptoms was only slightly earlier in the  
IL-12 treated mice (day 5), the subsequent progression to peak disease was accelerated with  
all mice displaying full hind limb paralysis by day 8. The duration of paralysis was also  
15 significantly prolonged lasting up to 14 days (range 11-14). Several mice treated with rmIL-  
12 that developed prolonged paralysis which persisted after the controls had fully recovered  
were sacrificed.

In a separate experiment, the effects of in vivo administration of rmIL-12 on  
disease severity was examined following the transfer of a suboptimal number of LNC  
20 ( $10 \times 10^6$  cells). Control mice receiving this lower number of LNC developed mild disease  
(Fig. 3b) with 1 out of 4 animals progressing to full hind limb paralysis and only minimal  
disease in the remaining 3 controls. In contrast, mice treated with rmIL-12 in vivo following

the transfer of  $10 \times 10^6$  LNC cells developed full clinical symptoms of disease with all mice scoring 2 or greater and 3 out of 4 mice progressing to full hind limb paralysis. The effects of rmIL-12 were also apparent after the transfer of as few as  $5 \times 10^6$  LNC cells with 3 out of 5 mice reaching a score of 1. At this cell number controls showed no signs of disease (data not shown).

**The effects of Anti-IL-12 antibody administration on the course of disease.**

To determine if endogenous IL-12 plays an essential role in disease transfer, mice were treated with  $200 \mu\text{g}$  of a sheep polyclonal antibody to murine IL-12 every other day for either 6 or 12 days following the transfer of  $30 \times 10^6$  PLP stimulated LNC cells. Controls received an equal amount of sheep IgG. The onset of clinical signs in the Sheep IgG treated controls was similar to that seen in untreated mice receiving PLP stimulated LNC (day 6-7, Fig. 4a). All control mice developed signs of disease graded 2 or greater (70% developed full paralysis). Administration of the anti-IL-12 antibody during the first 6 days after transfer did reduce the severity of disease, however, the onset of clinical signs was delayed by approximately 7 days. These mice subsequently went on to develop disease with all mice reaching a score of 2 or greater (80% developed full paralysis) with a similar time course of recovery to control animals. To determine if this delay of disease transfer could be sustained by a longer administration of anti-IL-12 antibody, we treated mice for 12 days following adoptive transfer of PLP primed LNC. Mice treated with anti-IL-12 antibody every other day for 12 days after the transfer of PLP stimulated LNC not only showed a more sustained delay in the kinetics of disease onset but also experienced dramatically

reduced clinical disease with only 2 out of 5 mice developing mild signs of disease (Fig. 4b).

### Example 2

NOD/LtJ mice (Jackson Laboratories) were treated with IL-12 to gauge the  
5 effect of the cytokine on an accepted animal model of insulin-dependent diabetes melitis  
(IDDM) [Kutani et al., Adv. Immunol. 51, 1992, p. 285]. Female NOD mice spontaneously  
develop an IDDM-like disease with destruction of the Beta cells in the pancreas and spilling  
of glucose into the urine beginning around 12-14 weeks of age. In the inventor's animal  
facility, female NOD mice show a disease incidence of approximately 88% by 30 weeks of  
10 age.

Female NOD mice were treated with two different protocols. In Treatment A,  
mice were given 10, 1 or 0.1  $\mu\text{g}$  (0.5, 0.05 or 0.005 mg/kg) murine IL-12 (mIL-12) i.p.  
three times a week for two weeks beginning at 9-11 weeks of age. In Treatment B, mice  
were given 1 or 0.1  $\mu\text{g}$  mIL-12 i.p. once a week beginning at 9 weeks of age and were  
15 continued on treatment until 25 weeks of age.

Mice under Treatment A receiving all three doses showed statistically  
significant decreases of disease incidence, with the 10  $\mu\text{g}$  dose being most effective (17%  
disease incidence) (see Table 1 and Fig. 5). Mice under Treatment B receiving 1  $\mu\text{g}$  weekly  
showed a large decrease in disease incidence (20%), while mice receiving 0.1  $\mu\text{g}$  did not  
20 show a measurable change in disease incidence (80%) (see Table 1 and Fig. 6).

Table 1

## INCIDENCE OF DIABETES IN IL-12 TREATED NOD MICE

age	unRx	IL-12 3X/week 2 weeks*			IL-12 weekly†	
		10µg	1µg	0.1µg	1µg	0.1µg
8					0/10	0/10
9					0/10	0/10
10	0/10	0/6	1/5	0/11	0/10	0/10
11	0/20	1/6	2/5	2/11	0/10	0/10
12	1/25	1/6	2/10	2/11	0/10	0/10
13	2/25	1/6	2/10	2/11	0/10	0/10
14	3/25	2/6	2/10	2/11	0/10	1/10
15	4/25	1/6	2/10	3/11	0/10	1/10
16	5/25	1/6	2/10	3/11	0/10	4/10
17	6/25	1/6	3/10	3/11	0/10	4/10
18	6/25	1/6	3/10	4/11	0/10	7/10
19	9/25	1/6	3/10	4/11	1/10	7/10
20	12/25	1/6	4/10	5/11	1/10	7/10
21	12/25	1/6	4/10	5/11	1/10	7/10
22	14/25	1/6	5/10	5/11	1/10	7/10
23	15/25	1/6	5/10	5/11	1/10	7/10
24	16/25	1/6	5/10	5/11	1/10	7/10
25	18/25	1/6	5/10	5/11	1/10	7/10
26	20/25	1/6	5/10	5/11	2/10	7/10
27	20/25	1/6	5/10	5/11	2/10	7/10
28	21/25	1/6	5/10	5/11	2/10	7/10
29	22/25	1/6	5/10	5/11	2/10	7/10
30	22/25	1/6	5/10	5/11	2/10	8/10

\* - treatment started at 9-10 weeks of age and continued for two weeks

† - treatment started at 9 weeks of age and continued for 15 weeks



All patent and literature references cited herein are incorporated by reference as if fully set forth.

What is claimed is:

1. A method for treating in a mammalian subject an autoimmune condition, said method comprising administering to said subject a therapeutically effective amount of an IL-12 antagonist.
2. The method of claim 1 wherein said autoimmune condition is promoted by an increase in levels of a cytokine selected from the group consisting of TNF- $\alpha$  or IFN- $\gamma$ .
3. The method of claim 2 wherein said autoimmune condition is selected from the group consisting of multiple sclerosis, systemic lupus erythematosus, rheumatoid arthritis, autoimmune pulmonary inflammation, Guillain-Barre syndrome, autoimmune thyroiditis, insulin dependent diabetes melitis and autoimmune inflammatory eye disease.
4. The method of claim 3 wherein said autoimmune condition is multiple sclerosis.
5. The method of claim 1 wherein said IL-12 antagonist is an antibody immunoreactive with IL-12.
6. The method of claim 5 wherein said antibody is administered in a dose of from about 0.05 to about 25 mg/kg.

7. The method of claim 5 wherein said antibody is administered in combination with a pharmaceutically acceptable carrier.
8. The method of claim 1 wherein said antagonist is administered in a dose of about 0.05 to about 25 mg/kg.
9. The method of claim 1 wherein said antagonist is administered in combination with a pharmaceutically acceptable carrier.
10. A method for treating in a mammalian subject an autoimmune condition, said method comprising administering to said subject a therapeutically effective amount of IL-12.
11. The method of claim 10 wherein the development of said autoimmune condition is promoted by an increase in levels of a cytokine selected from the group consisting of TNF- $\alpha$  or IFN- $\gamma$ .
12. The method of claim 11 wherein said autoimmune condition is selected from the group consisting of multiple sclerosis, systemic lupus erythematosus, rheumatoid arthritis, autoimmune pulmonary inflammation, Guillain-Barre syndrome, autoimmune thyroiditis, insulin dependent diabetes melitis and autoimmune inflammatory eye disease.

13. The method of claim 12 wherein said autoimmune condition is insulin-dependent diabetes melitis.

14. The method of claim 10 wherein said IL-12 is administered at about 0.001 to about 1000  $\mu\text{g/kg}$ .

15. The method of claim 10 wherein said IL-12 is administered in combination with a pharmaceutically acceptable carrier.

Fig 1

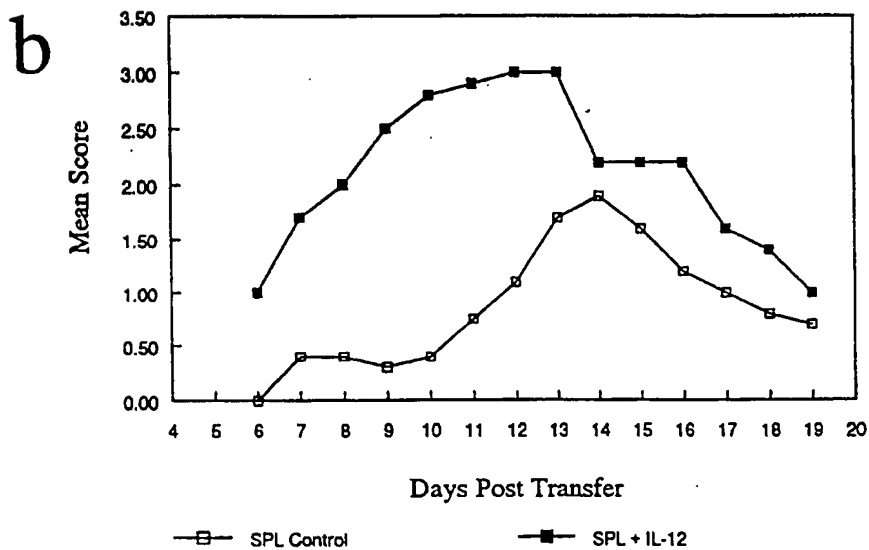
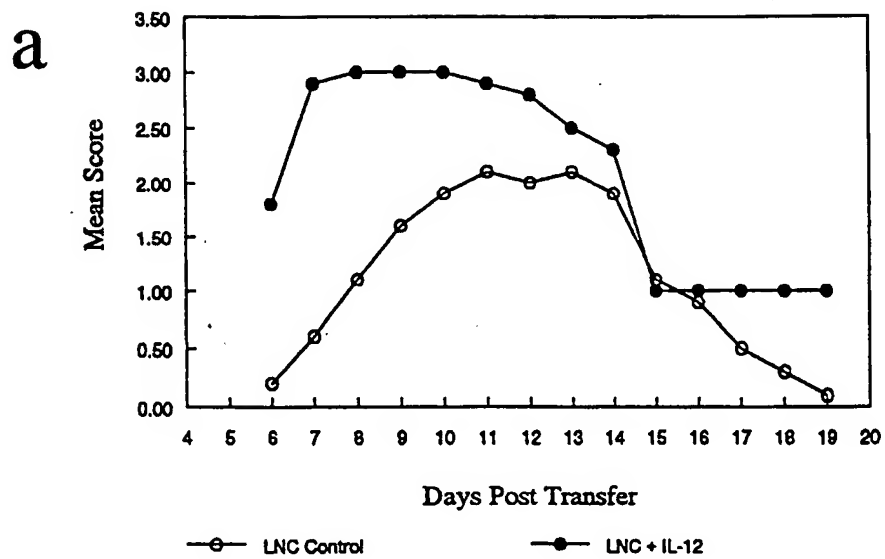


Fig 2

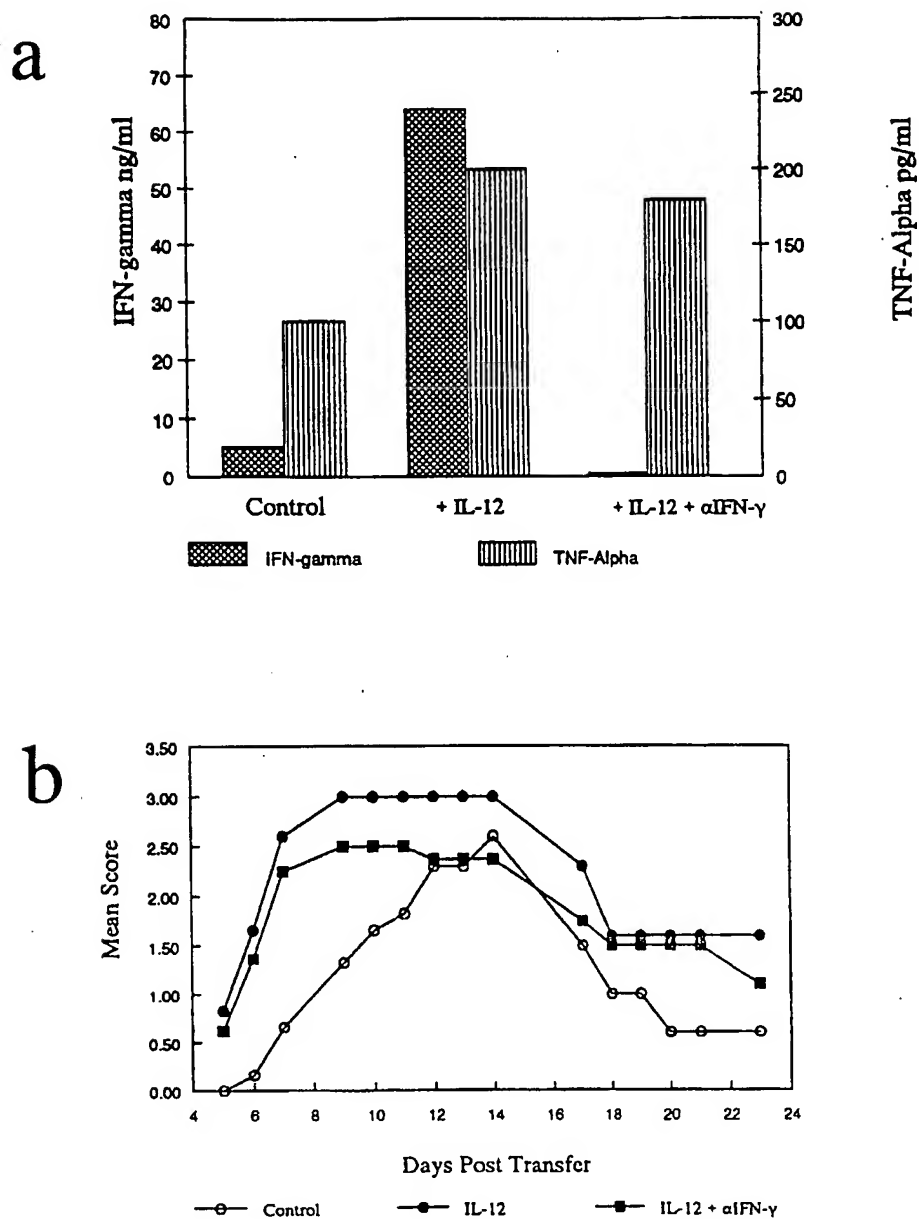


Fig 3

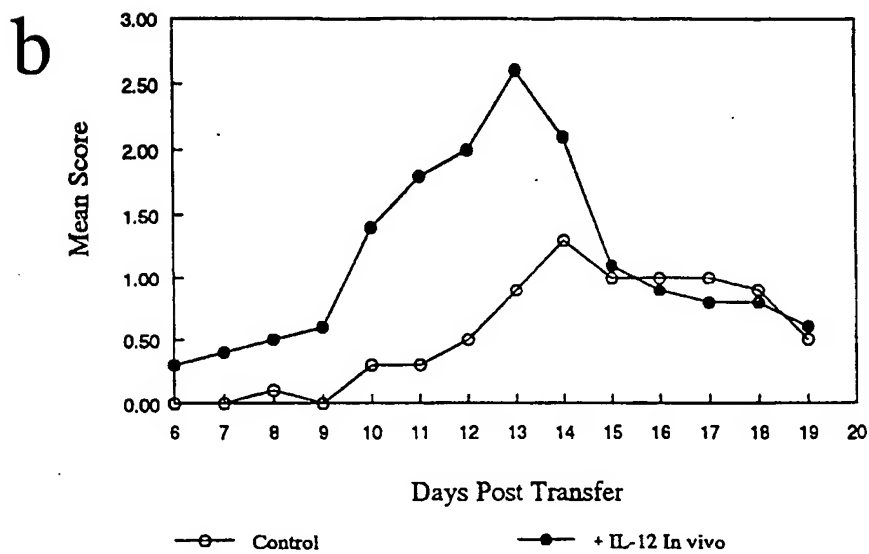
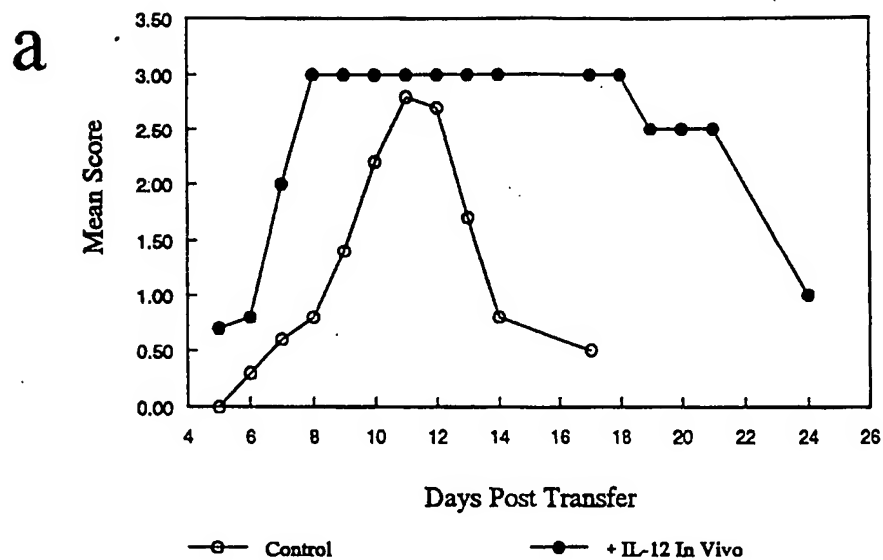


Fig 4

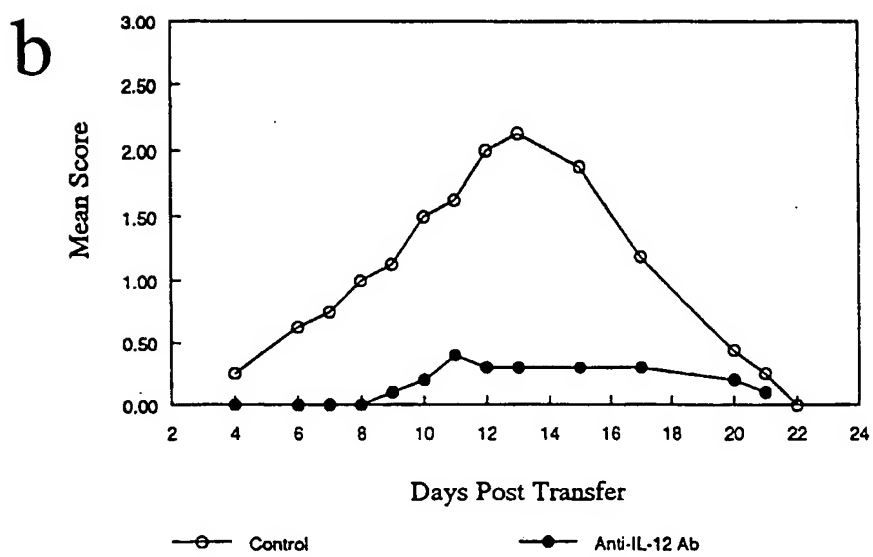
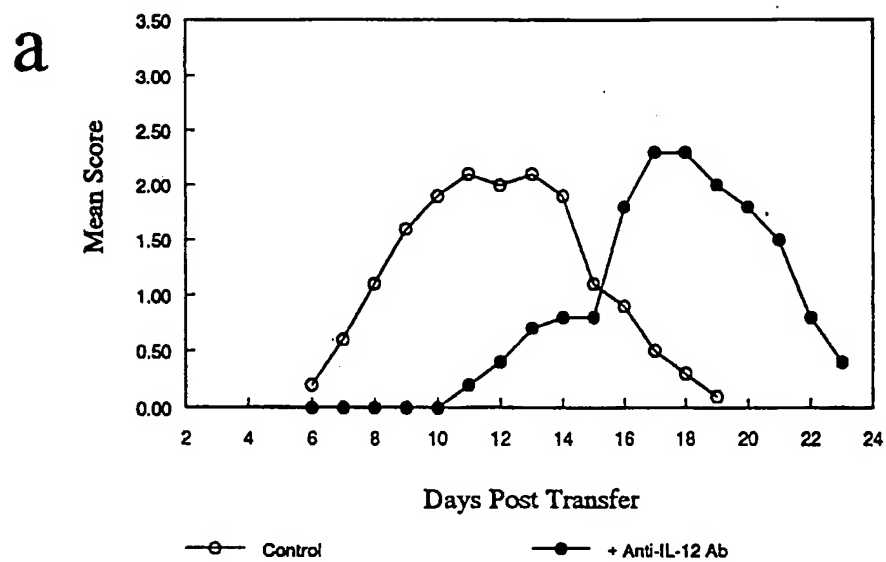




Fig. 5

Protocol A

IL-12/NOD disease incidence combined data

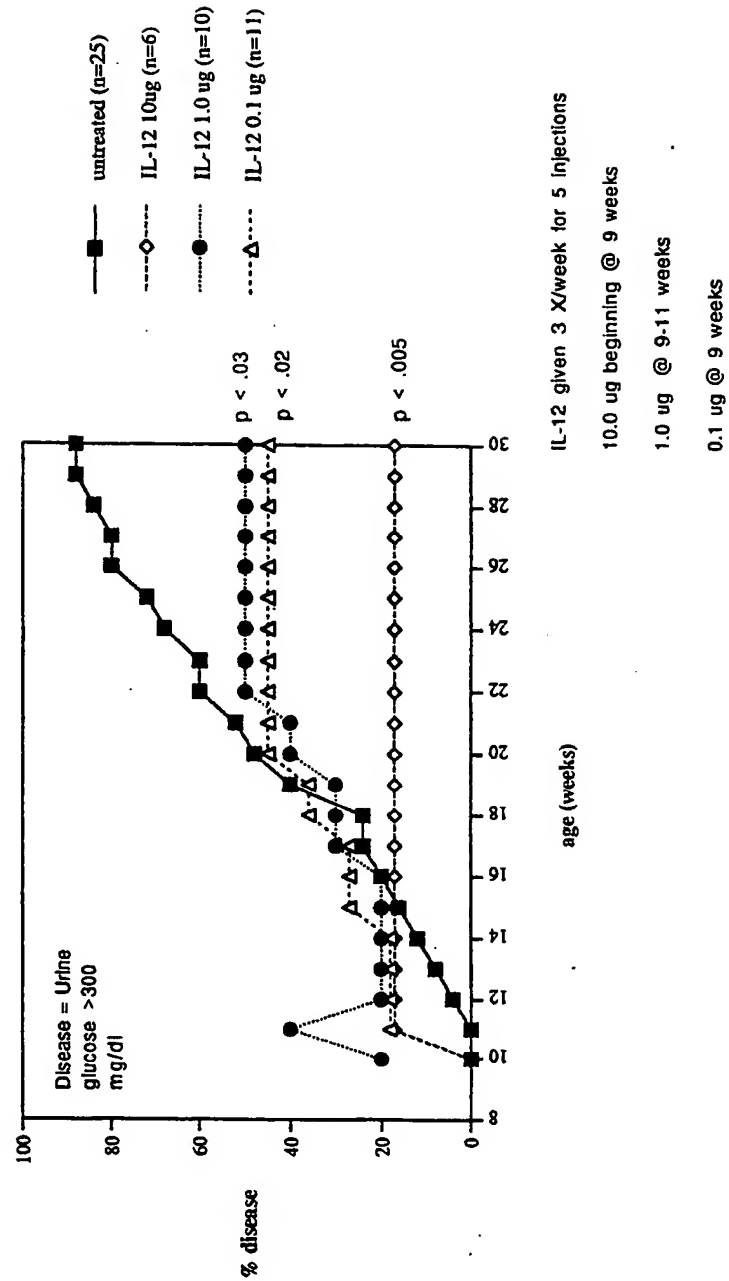
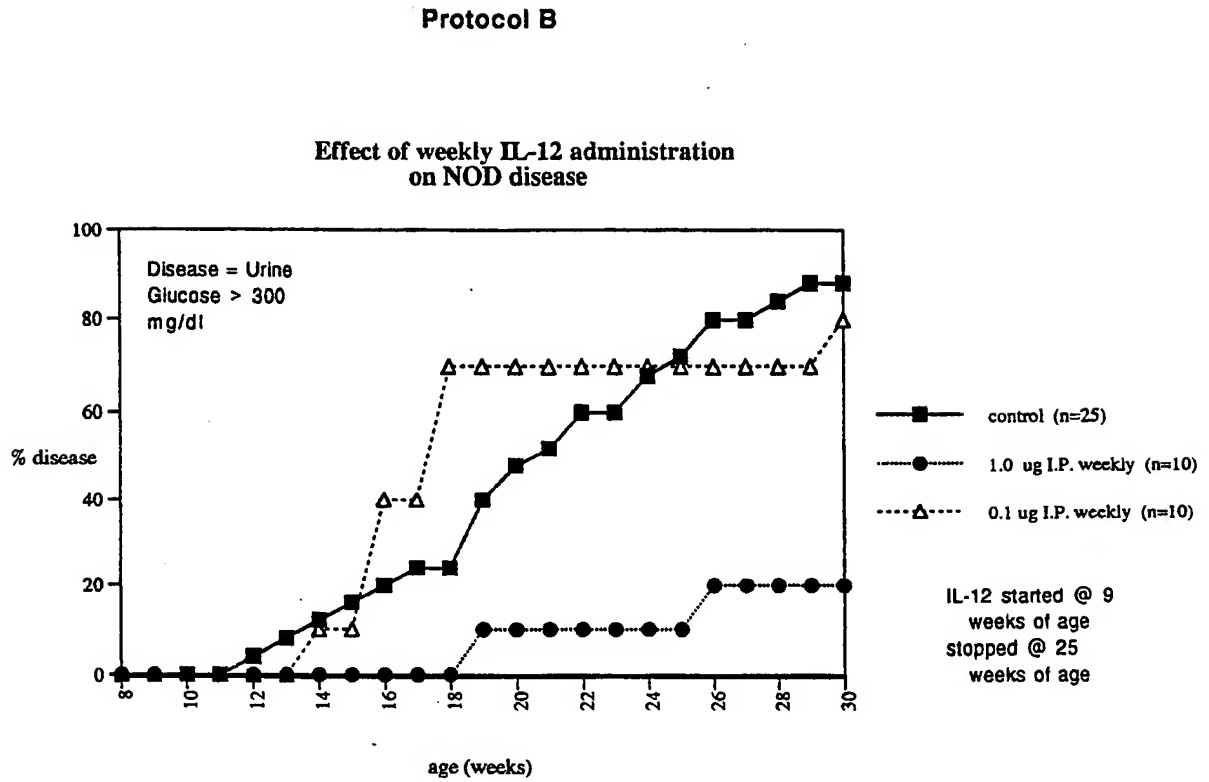


Fig. 6



# INTERNATIONAL SEARCH REPORT

Int. Application No  
PCT/US 95/02550

**A. CLASSIFICATION OF SUBJECT MATTER**  
IPC 6 A61K38/20 A61K39/395

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)  
IPC 6 A61K C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO,A,92 05256 (GGENETICS INSTITUTE) 2 April 1992 cited in the application see the whole document ---	1-15
A	EP,A,0 433 827 (HOFFMANN-LA ROCHE) 26 June 1991 cited in the application see the whole document ---	1-15
A	THE JOURNAL OF EXPERIMENTAL MEDICINE, vol. 173, no. 4-94, April 1991 pages 869-879, CHAN S.H. ET AL. 'Induction of Interferon gamma Production by Natural Killer Stimulatory Factor ...' see the whole document ---	1-15
-/--		

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

\* Special categories of cited documents :

- \*A\* document defining the general state of the art which is not considered to be of particular relevance
- \*E\* earlier document but published on or after the international filing date
- \*L\* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- \*O\* document referring to an oral disclosure, use, exhibition or other means
- \*P\* document published prior to the international filing date but later than the priority date claimed

\*T\* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

\*X\* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

\*Y\* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

\*G\* document member of the same patent family

Date of the actual completion of the international search

14 June 1995

Date of mailing of the international search report

21-06-1995

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2  
NL - 2280 HV Rijswijk  
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,  
Fax (+31-70) 340-3016

Authorized officer

Moreau, J

# INTERNATIONAL SEARCH REPORT

Inte al Application No  
PCT/US 95/02550

**C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT**

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	<p>THE JOURNAL OF EXPERIMENTAL MEDICINE, vol. 181, January 1995 pages 381-386, LEONARD J.P. ET AL. 'Prevention of Experimental Autoimmune Encephalomyelitis by Antibodies against Interleukin 12' see the whole document -----</p>	1-15

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 95/02550

**Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:  
**Remark : Although claims 1-15 are directed to a method of treatment of the human/animal body the search has been carried out and based on the alleged effects of the compound/composition.**
2. ☐ Claims Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

**Remark on Protest**

- ☐ The additional search fees were accompanied by the applicant's protest.  
☐ No protest accompanied the payment of additional search fees.

# INTERNATIONAL SEARCH REPORT

Information on patent family members

Int. Application No

PCT/US 95/02550

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A-9205256	02-04-92	AU-A- 8723691 EP-A- 0549711 JP-T- 6501009	15-04-92 07-07-93 27-01-94
EP-A-433827	26-06-91	AU-B- 5471294 AU-A- 6834990 JP-A- 5294999	09-06-94 27-06-91 09-11-93

Form PCT/ISA/210 (patent family annex) (July 1992)